METHODS FOR FORENSIC DNA QUANTITATION

RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Patent Application Ser. No. 61/268,770, filed Jun. 15, 2009, entitled "Improved Methods for Forensic DNA Quantitation," by Selden et al., which is incorporated herein by reference.

GOVERNMENT FUNDING

The U.S. Government has a paid-up license in this invention and the right in limited circumstances to require the patent owner to license others on reasonable terms as provided for by the terms of (Grant No. NIJ 2008-DN-BX-K009) awarded by the National Institutes of Justice, Office of Justice Programs, US Department of Justice.

FIELD OF INVENTION

Described herein are inventive methods and devices for nucleic acid quantification and, in particular, to microfluidic methods and devices for nucleic acid quantification.

BACKGROUND

Nucleic acid quantification is a critical or desirable step in a wide variety of assays and applications. For example, nucleic acid quantification is an important step in human forensic identification. For example, short tandem repeat (STR) analysis of DNA is often based on a multiplexed PCR assay, and such assays are generally most reliable within a narrowly defined range of sample DNA concentration. If too little sample DNA is used in the assay, artifacts including allele peak height imbalance and allele drop-out can occur. If too much sample DNA is used, artifacts including increased stutter, non-specific band creation, incomplete non-template addition, and pull-up peaks resulting from incomplete color separation can occur. These artifacts can lead to difficulties in interpretation of an STR profile but can be mitigated by using an appropriate amount of sample DNA. In another example, forensic casework samples have the potential to be contaminated with non-human mammalian, bacterial, or fungal DNA which, when present, contributes to the total DNA in the sample. Accordingly, for evaluation of crime scene samples, the DNA Advisory Board to the FBI recommends the use of human-specific quantification rather than total DNA quantification, which can ensure that an appropriate amount of human DNA is subjected to amplification even if bacterial, fungal, or other non-human DNA is present is the sample.

SUMMARY OF THE INVENTION

Described herein are inventive methods and devices for nucleic acid quantification and, in particular, to microfluidic methods and devices for nucleic acid quantification.

In one aspect, a method for quantifying a target nucleic acid in a sample fluid containing or suspected of containing the target nucleic acid is provided. The method comprises combining in a microfluidic channel the sample fluid and a binding agent comprising a signaling moiety, wherein the binding agent becomes immobilized with respect to the target nucleic acid, to form a test fluid, locating the test fluid in a detector region in the microfluidic channel, detecting the signaling moiety, and quantifying the target nucleic acid in the sample fluid within 1 hour of combining the sample fluid and the binding agent.

In another aspect, a method for quantifying a target nucleic acid in a sample fluid containing or suspected of containing the target nucleic acid is provided. The method comprises combining in a microfluidic channel the sample fluid and a binding agent comprising a signaling moiety, wherein the binding agent becomes immobilized with respect to the target nucleic acid, to form a test fluid, locating the test fluid in a detector region in the microfluidic channel, detecting the signaling moiety, and quantifying the target nucleic acid in the sample fluid, wherein the target nucleic acid has a concentration less than 1 nanograms per microliter or is present in a total amount in the sample fluid of less than 1 nanogram.

In still another aspect, a method for quantifying a target nucleic acid in a forensic sample fluid containing or suspected of containing the target nucleic acid is provided. The method comprises combining in a microfluidic channel the forensic sample fluid and a probe fluid containing a binding agent comprising a signaling moiety, wherein the target nucleic acid in the forensic sample fluid has not been amplified, locating the combined fluids in a detector region in the microfluidic channel, detecting the signaling moiety, and quantifying the target nucleic acid in the sample fluid.

In yet another aspect, a method for quantifying a target nucleic acid in a sample fluid containing or suspected of containing the target nucleic acid and also containing a contaminating non-human nucleic acid is provided. The method comprises combining in a microfluidic channel the sample fluid and a probe fluid containing a binding agent comprising a signaling moiety, wherein the target nucleic acid in the sample fluid has not been amplified, locating the combined fluid in a detector region in the microfluidic channel, detecting the signaling moiety, and quantifying the target nucleic acid in the sample fluid.

In still another aspect, a method for quantifying of a target nucleic acid in a sample fluid containing or suspected of containing the target nucleic acid is provided. The method comprises providing a microfluidic device coupled to an electronic device comprising a detector comprising an integrated laser, combining in a microfluidic channel of the microfluidic device the sample fluid and a binding agent comprising a signaling moiety, wherein the binding agent becomes immobilized with respect to the target nucleic acid, locating the combined fluid in a detector region in the microfluidic channel positioned in operative proximity to the detector of the electronic device, irradiating the signaling moiety using the integrated laser, and quantifying the target nucleic acid in the sample fluid.

In yet another aspect, a method for manipulating a target nucleic acid in a sample fluid containing the target nucleic acid is provided. The method comprises providing a microfluidic device comprising a plurality of microfluidic channels and active areas for sample manipulation coupled to an electronic device comprising a detector, combining in a microfluidic channel of the microfluidic device the sample fluid and a binding agent comprising a signaling moiety, wherein the binding agent becomes immobilized with respect to the target nucleic acid, locating the combined fluid in a detector region in the microfluidic channel positioned in operative proximity to the detector of the electronic device, quantifying the target nucleic acid in the sample fluid, and directing a selected quantity of the sample fluid to an active